



said solid support is a membrane strip and said polypeptides are coupled to the membrane in the form of parallel lines. It has to be understood that certain peptides, or antibodies as defined above, alternatively, are not attached to a solid support but are provided in the binding solution to be used as competitors and/or to block other antibodies that are present in sera from patients with autoimmune diseases other than SLE, thereby decreasing or eliminating possible cross-reaction and/or a specific binding.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1: HPLC profile of the Endo-Lys digest.

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Figure 2: Immunodot of HPLC fractions with 5 patients sera and 1 control serum.

Figure 3: Immunodot of the C-terminal peptide (C-term mod) and without (C-term nt mod) dimethylarginine, and of the recombinant (baculo SmD, coli SmD) and natural protein (native). Strips were incubated with a anti-SmD positive serum (+) and a control serum (-). Total protein staining (Aurodyne) was performed on the third strip.

Figure 4: LIA with modified (dimethyl arginine) C terminal peptide (fraction 15 from EndoLys-C digest, line 1 on the strip), and non-modified C terminal peptide (fraction 8 from the EndoLys-C digest, line 2 on the strip), both applied in equal amounts (60 ng). Additionally, 7, 15 and 30 ng of recombinant SmDI from baculovirus- or E. Coli-infected insect cells (resp. 4,5,6 and 7,8,9) as well as 15 and 30 ng of a mixture of gel-purified SmD (native) were applied to the strips. The total protein staining (Aurodyne) was performed on the first strip. The strips were incubated with (A) a panel of anti-SmD positive sera selected by INNO-LIA ANA from ANF-positive sera, (B) a panel of anti-SmD positive sera selected by INNO-LIA ANA from a cohort of SLE patients diagnosed according to the ACR criteria, (C) sera selected from MCTD patients (control panel) and (D) sera selected from ANF- negative sera (control panel). No reactivity was observed with sera from the control panels.

We have demonstrated for the first time that well defined secondary modifications (mostly N^G, N^G -dimethylarginine) are present on the Arg residues of the C-terminal peptide, that are followed by a glycine residue. Moreover, we have raised evidence that the C-terminal peptide can only show an immunoreactivity almost identical to the immunoreactivity of natural



30 SmD, if these arginine residues are methylated. These dimethylarginines present on the nine Arg positions of the C-terminus, have been demonstrated for the first time in the natural SmDl molecule. In SmD2 no dimethylarginine was retrieved while in the C-terminus of SmD3 the four RG motifs in the C-terminus again were found to be dimethylated.

35 The amino acid N^G, N^G -dimethylarginine is the result of a post-translational modification which seems to occur predominantly in RNA binding proteins (Najbauer, 1993). These nuclear proteins are enzymatically modified by a nuclear protein methylase 1 (S-adenosyl-methionine: protein-arginine N-methyltransferase, E.C.2.1.1.23; Rajpurohit, et al.,1994). The structural specificity of this enzyme seems to be an arginine containing peptide with glycine in the C-flanking position as was shown by substrate evaluation with synthetic peptides (Rawal, 1995). Nevertheless, in the same study it was demonstrated that the entire molecule also plays an
40 important though thus far unknown role in the methylation process. Interestingly, this cellular methylation process can be mimicked *in vitro* with purified methylase1 as was illustrated with recombinant heterogeneous nuclear RNP protein A1 (Rajpurohit, et al. 1994) .

From our results, we thus can conclude that in SmD immunoreactivity, at least 2 epitopes are involved. One of the epitopes is apparently present in the recombinant SmDl molecule and
45 can not be assigned to a linear epitope (epitope

immobilized on the membrane will preferentially be the methylated and unmethylated form of poly(Arg-Gly), combined with native and thus methylated SmDl and/or SmD3 and/or Sm69, and unmethylated, recombinant SmDl and/or SmD3 and/or Sm69.